Electrospun gelatin-nanocontainers for enhanced biopharmaceutical performance of piroxicam: *in vivo* and *in vitro* investigations

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Abstract

Background: Piroxicam exhibits low oral bioavailability due to its meagre solubility in water. The intent of the study described below was to ameliorate bioavailability of the drug by employing a solubility-enhancing encapsulation technique.

Methods: Seven samples were formulated with piroxicam and gelatin using both the solvent evaporation method and electrospraying method together. Evaluation of solubility and release rate in water, and assessment of bioavailability in rats were carried out in comparison with piroxicam plain drug powder (PPDP). Other *in vitro* explorations were accomplished using powder X-ray diffraction (PXRD) analysis, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy.

Results: All the piroxicam-loaded gelatin-nanocontainers (PLGNs) enhanced solubility and release of the payload in water. In particular, a PLGN formulation consisting of piroxicam and gelatin at 1/8 (w/w) ratio presented about 600-times greater solubility of the drug than that shown by PPDP. Moreover, 85.12 ± 10.96% payload was released from this formulation in 10 min which was significantly higher than that dissolved from PPDP in 10 min (11.81 ± 5.34%). The drug content, drug loading and encapsulation efficiency of this formulation were 93.41± 0.56%, 10.45± 0.06% and 66.74± 6.87%, respectively. The drug loaded in PLGNs existed in the amorphous state as confirmed by XRD and DSC analyses, and it was more stable when analyzed by TGA. Moreover, FTIR spectroscopic analysis suggested non-existence of any piroxicam-gelatin interaction in the formulation. In the SEM image, PLGNs appeared as round-shaped and smooth-surfaced particles, exhibiting a particle size of <1000 nm. Amelioration in bioavailability of piroxicam with the above-mentioned PLGN formulation was 4-fold as compared to that with PPDP.

Conclusion: The PLGN formulation fabricated with piroxicam and gelatin at 1/8 (w/w) might be a promising system for enhanced biopharmaceutical performance of the drug.

Keywords*:* aqueous solubility; electrospraying; gelatin encapsulation; nanocontainers; oral bioavailability; piroxicam

Introduction

Piroxicam, an anti-inflammatory and analgesic substance, is prescribed to mitigate pain and associated inflammation. In particular, it is useful in the treatment and management of pain accompanied by rheumatoid arthritis and osteoarthritis.[1] It impedes the activity of both cyclooxygenase (COX) type I and COX type II enzymes; therefore, formation of prostaglandins (PGs) is ceased.[2] PGs are a group of physiologically active lipids which induce pain and agony. Some interactions of piroxicam with other drugs have been reported as well. For example, when administered along with cadmium, it exhibits more toxicity.[3] On the other hand, when co-administered with rosuvastatin, its adverse effects such as peptic ulceration and hepatorenal damage are mitigated.[4]

Piroxicam is a member of class II of Biopharmaceutics Classification System (BCS) of drugs.[5] The substances included in this class are either completely insoluble or meagrely soluble in the aqueous fluids; however, they possess remarkable ability to traverse cell membranes efficiently.[6] Piroxicam is scarcely soluble in the aqueous media as it imparts about 23 µg/mL solubility in water.[7] Water is the main component of gastrointestinal (GI) fluid. A BCS class II entity is meagrely dissolved in GI fluid present in the lumen of the GIT. Only the drug in solution state in GI fluid can traverse cells.[8] Owing to exiguous solubility in GI fluid, piroxicam is not sufficiently permeated through cells; hence, the drug titer in the systemic circulation is not effective to block the activity of COX type I and COX type II enzymes well. An alternative way to achieve the effective bioavailability is to administer the drug at higher quantity; however, this may induce severe deleterious effects in the GIT. Thus, adoption of a suitable solubility improving technique is a more sagacious way to cope with this problem.

Solubility of a BCS class II substance can be ameliorated via several techniques such as

fabrication of nano-sized drug-loaded hydrophilic particles, dispersion of a drug in hydrophilic polymers, association of a drug with cyclodextrins, wrapping a drug payload core in a hydrophilic wall material, casting of drug-laden porous silica particles, self-emulsifying systems, microemulsions, co-crystallization and others.[6,9-11] Pharmaceutical nanoencapsulation is a promising technology for resolving poor solubility and bioavailability problems of a BSC class II agent. It is an approach to enwrap a solid, liquid or gaseous substance in diminutive polymeric capsules.[12] In this drug delivery system, the wrapper or wall material protects the core from the surrounding environment, and storage stability of the payload can be extended.[13] Gelatin, a heterogeneous mixture of chains of amino acids, is soluble in warm water. It is derived from collagen protein by hydrolysis. It is broadly availed in pharmaceutical, food and cosmetic industries owing to its absolutely non-toxic, biodegradable, ecofriendly, biocompatible and high payload holding characteristics. Also, it is convenient to administer and eliminates appropriately in physiological milieu.[14] It is a regularly utilized wrapping or wall material for encapsulation of pharmacologically active substances and nutrients.[15] A number of previous studies have shown that gelatin can foster the aqueous solubility and oral bioavailability of numerous drugs.[9,16] A hydroalcoholic compound solution of a BCS class II drug and gelatin can be transmuted to a dry particulate form by the electrospraying technique. In this process, water is evaporated to a great deal and the ethanolic solution of the drug (core) is enwrapped or encapsulated by gelatin (shell or wall).[12,17] Electrospraying is an excellent technique to obtain drug-loaded spherical gelatin nanocapsules.[18] A pharmaceutical nanocapsule is a nano-sized (< 1 μm) drug-loaded spherical particle having a core-shell morphology.[19]

The aim of this work was to augment the aqueous solubility and release rate of piroxicam using gelatin nanoencapsulation method. Thus, PLGNs were prepared by the electrospraying

technique. Solubility and release rate of piroxicam in PLGNs were investigated. The crystalline physiognomies were determined using PXRD and DSC methods. TGA was executed for measuring percent weight loss occurring in a sample owing to gradually rising temperature. FTIR spectroscopic analyses were carried out for exploring possible interlinkage between piroxicam and gelatin. The shapes and surface attributes of particles were inspected using a SEM. Oral bioavailability was evaluated in white albino laboratory rats (Sprague Dawley rats). Area under the curve (AUC), the highest drug titer in plasma (C_{max}) and time taken from dose administration to the appearance of C_{max} (T_{max}) were used as parameters for assessing bioavailability.

Materials and methods

Materials

Piroxicam (purity ≥ 98%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Gelatin was procured from Daejung Chemical Co. (Siheung, South Korea). All other chemicals and solvents were of reagent grade.

Preparation of PLGNs

All the formulations were prepared via the electrospraying technique. The recipe of samples prepared in this study is shown in Table 1. For each sample, 100 mg PPDP was completely dissolved in 60 mL ethanol at 40°C (solution *A*). Gelatin was dissolved separately in 90 mL distilled water at 40°C (solution *B*). Then, both the solutions were mixed together thoroughly to get a final clear solution (solution *C*). The quantities of solvents in solution *A* and solution *B* were taken so that the composition of solvent in solution *C* was approximately 40% hydroalcoholic mixture. Solution *C* was sprayed under the influence of a high voltage using an electrospraying machine (Model ESR 100, NanoNC equipment; Seoul, South Korea). The compound solution to

be electrospun was filled in the barrel of a syringe made up of glass (Hamilton Co., Reno, NV, USA). The solution-loaded syringe was properly attached to the syringe holder. The plunger of the syringe was pushed by the pump to move the solution via a single-lumen nozzle at a constant rate of 1 mL/h. At the voltage of 12.5 kV, Taylor cone was formed and the jet was converted to plume beneath the Taylor cone. Each electrospun sample was collected and placed at 40°C until constant weight, and stowed in air-tight microtubes.

Solubility of piroxicam in PLGNs

For each PLGN formulation, excess of dried product was poured into a 2 mL capacity Eppendorf microtube containing 0.5 mL distilled water and mixing was done for a minute using a vortex-mixer. Then, microtubes were swayed (100 rpm) for 5 days in a water-bath (37°C). After 5 days, the samples were centrifuged at 7000 ×g for 5 minutes. In another clean microtube, 0.2 mL of clear supernatant was poured and diluted with equal quantity of acetonitrile. The diluted sample (10 µL) was analysed by a *1260-Infinity* HPLC equipment (Agilent Technologies, CA, USA) using a *Quasar*^{TM} C18 column (4.6 mm I.D. × 150 mm, 5 µm) (PerkinElmer, Shelton, CT, USA) set at 40°C. The mobile phase was comprising of trifluoroacetic acid and acetonitrile (60:40, v/v), which eluted at a rate of 1 mL/min. The eluent was quantified at 332 nm for the determination of piroxicam concentration.[20]

Content of piroxicam in PLGNs

A PLGN formulation, equivalent to 5 mg piroxicam, was transferred to a 100 mL volumetric flask containing 100 mL of 40% aqueous ethanol. The lid was tightly closed to minimize the evaporation of the solvent. Then, the flask was secured in a water-bath (37°C) for 2 hours. The solution was cleared by magnetic stirring. Thus, a stock solution of 50 µg/mL drug concentration was obtained. This clear solution was filtered (0.45 µm) and aptly diluted with

acetonitrile. The dilution was analysed in accordance with the above-mentioned HPLC method for determining actual concentration of the drug. The content of piroxicam in each PLGN sample was computed using the following formula: $P_C = P_A/P_T \times 100$. Where, P_C stands for piroxicam amount in the sample, P_A stands for actual quantity of piroxicam in the diluted sample, and P_T stands for the theoretical quantity of the dilution. For each composition, the test was performed in triplicate (n = 3).

Drug loading

The percentage of piroxicam loading was computed by using the following formula: $P_L = P_W/P_N \times 100$. Where, P_L is the percentage of piroxicam loading to PLGN, P_W is the weight of piroxicam in grams present in P_N of PLGN, and P_N is the quantity of PLGN in grams.

Encapsulation efficiency

The optimized PLGN formulation, equivalent to 5 mg drug, was placed in 2 mL capacity *Amicon® ultra-2* centrifugal filter (UFC200324, MWCO 3000 *Da*, Merck Millipore, Billerica, MA, USA) and rinsed thrice with absolute alcohol, each time with 1 mL volume. The filtrate was discarded each time. The ultracentrifugal filtration was accomplished with 7000 ×g RPMs for 10 min. Then, the residue was dissolved gradually with 40% hydroalcoholic solution which was preheated at 50°C. Each time, 1 mL volume was used until complete dissolution. The filtrate was preserved in 10 mL capacity measuring flask each time. Then, the volume was made 100 mL with ethanol. The theoretical concentration of this solution was 50 µg/mL. The actual concentration of piroxicam in this solution was determined by the HPLC method as described above. The encapsulation efficiency of PLGN was calculated by using following formula: $E_E = P_E/P_T \times 100$, where, E_E is the encapsulation efficiency, P_E is the actual quantity in grams of encapsulated piroxicam, and P_T is the total grams of piroxicam present in PLGN in accordance with the drug

content.

Release rate of piroxicam from PLGNs

Release rate of piroxicam from a PLGN formulation was determined using the basket apparatus (Model Vision Classic 6, Hanson Research Co., CA, USA). A PLGN formulation corresponding to 40 mg piroxicam was sealed in a dialysis pouch (12K-14K *Da*). Then, the sample-loaded pouch was encaged in the basket by fitting the basket with the shaft. In order to sink the basket in 900 mL of 2% (w/v) aqueous solution of Tween 80 filled in the vessel below, the rotating shaft (100 rpm) was pulled down. The temperature of the release medium was already maintained at 37 ± 0.5°C. After each predetermined time interval, 1 mL of release medium was sampled using a syringe, and filtered into an HPLC vial using a syringe filter (0.22 μm). Piroxicam concentration was quantified by the above-mentioned HPLC method. For each PLGN formulation, experiment was done in sextuplicate (n = 6).

X-ray diffraction

A *D-MAX 2500PC* X-ray diffractometer (Rigaku Corporation, Tokyo, Japan), using a $CuK\alpha_1$ monochromatic emission source, was used for determining the degree of crystallinity or amorphousness of the samples. The analyses were carried out in the range of 5°-50° at a scanning speed of 10°/min with a step-size of 0.02°/sec and scanning mode set at 2θ. The current flow and voltage supply were regulated at100 mA and 100 kV, respectively.

Differential scanning calorimetry

A *DSC-Q20* differential scanning calorimeter (TA Instruments Co.; New Castle, DE, USA) was employed for confirming the crystalline or amorphous status and the other thermal physiognomies of the samples. About 5 mg quantity of a sample to be tested was placed and sealed in a *Tzero* aluminum crucible (TA Instruments Co.; New Castle, DE, USA). The sealed sample

was placed in the calorimeter which was catered with a constant supply of nitrogen (30 cm^3/min). The scanning was performed by heating the sample in the range of 30°-300°C at the rate of 10°C/min.

Thermogravimetric Analysis

The decrease in weight of a sample occurring with gradual raise in temperature was determined by a *TGA Q50* TG analyzer (TA Instruments Co.; New Castle, DE, USA). About 5 mg quantity of a sample, put into the hangable platinum crucible, was lowered into the furnace by the automatic moving arm. The sample enclosed in the furnace was gradually heated in the range of 50-550°C at the heating speed of 10°C/min and in the constant supply of nitrogen (25 cm^3/min). The decrease in weight (%) of the sample was noted.

Scanning electron microscopy

An *S-4800* scanning electron microscope (Hitachi, Japan) was employed for studying the shape, size and surface quality of the particles. The samples to be inspected were clung on the exposed surface of a double-side adhesive tape affixed to a brass stub. Then, the samples were coated with platinum (Emitech K-575-K Ion-Sputter Coater) under 8×10^{-3} mbar vacuum. The current supply and turbo speed settings were adjusted at 20 mA and 90%, respectively. The coated samples were scanned by SEM and images were recorded.

Fourier transform infrared spectroscopy

FTIR spectra of the samples were recorded in the range of 4000-400 cm^{-1} at the resolution of 2 cm^{-1} using a *Nicolet-6700* FTIR spectrophotometer (Thermo Scientific, PA, USA).

Bioavailability assessment in rats

Acclimatization of animals – Twelve white albino laboratory rats (Sprague Dawley rats), each of 260-300 g body weight, were divided into two equal groups. They were kept in cages

placed under controlled environment of 21-28°C temperature and 45-65% relative humidity. Before giving a dose of PPDP or PLGN formulation via the oral route, each one was fasted for 1.0-1.5 day; nevertheless, access to drinking water was not discontinued. All the steps involved in exploitation of animals as experimental organisms in research were conducted strictly following the 'Guiding Principles in the Use of Animals in Toxicology' recommended by the Society of Toxicology, and NIH policy regarding the animal welfare. All the protocols were also reviewed by the Institutional Animal Care and Use Committee (IACUC) of Government College University Faisalabad prior to granting the approval of the study (Ref. No. GCUF/ERC/2138).

Arterial intubation for blood sampling – Each fasted animal was anesthetized by injecting a mixture of tiletamine and xylazine (2/1, v/v) intramuscularly. Cannulation of a polyethylene tube into the right carotid artery was accomplished surgically. The tube was passed through the hollow spring attached with the infusion harness in order to protect it from likely damage by the animal. Each harnessed rat was kept in a separate adequate-sized cage where it was able to move freely.

Dose administeration – A dose of PPDP or optimized PLGN formulation, corresponding to 7 mg/kg weight, was suspended in 700 µL water and promptly given to a harnessed rat through the oral route using an oral gavage.

Blood collection, plasma separation and storage – A 200 µL aliquot of blood was withdrawn from the carotid artery of a cannulated rat with the help of 1 mL capacity heparinized syringe at each of the following time points: 1, 2, 3, 4, 5, 6, 12, 24, 36 and 48 h. Plasma was separated tout de suite by centrifugation at 5000 ×g for 10 min. Subsequently, the plasma samples were preserved at -20°C until further experimentations.

Extraction and HPLC analysis – A 50 µL plasma quantity was poured in a 2 mL capacity microtube and an aliquot (20µg/ml) of naproxen sodium was added to it as an internal standard. Subsequently, 700 µL of acetonitrile were added to it as a menstruum for liquid-liquid extraction.

After a thorough mixing, centrifugation was performed at 5000 ×g for 3 min. Then, the clear supernatant was carefully shifted to a small volume HPLC vial and analyzed in accordance with the method as described above.

Calculation of pharmacokinetic parameters – The pharmacokinetic parameters, such as area under the curve (AUC, h.µg/mL), the highest piroxicam titer in plasma (C_{max}, µg/mL) and time taken from dose administration to the appearance of C_{max} (T_{max}, h), were computed employing a non-compartmental analysis (*PK* Solver, version 2.0, Pharsight Co., CA, USA) following linear trapezoidal method.[21] Corresponding to each time point, the level of significance (*p*-value) between two means of piroxicam concentrations was determined using the Student *t*-test. In general, a *p*-value less than 0.05 suggests presence of a significant difference. On the other hand, a *p*-value more than 0.05 represents a nonsignificant difference.

Results and discussion

Gelatin is a hydrophilic polypeptide which has been used extensively in encapsulation of BCS class II drugs so as to enhance their solubility and oral bioavailability.[12,22] Polymeric nanoparticulated drug delivery systems also improve solubility, dissolution rate and oral bioavailability of such drugs.[9,23] A drug-laden entity owning measurements of less than 1 µm is considered to be a pharmaceutical nanoparticle.[19] Electrospraying technique is an awesome way to obtain drug-loaded polymeric nanoparticles.[8,23] Thus, in this research PLGNs were prepared using the electrospraying technique. A recipe of each nanocontainer sample is given in Table 1.

The outcomes of the aqueous solubility of piroxicam in PLGNs are shown in Fig. 1. Solubility of piroxicam in samples I-IV was augmented as the drug/gelatin ratio increased to 1/8 (w/w). This was owing to enhanced wettability of the drug by hydrophilic gelatin. As the drug/gelatin ratio increased in samples V-VII from 1/10 (w/w) to 1/20 (w/w), an apparent reduction in solubility was noticed. Even so, the solubility outcomes were not significantly different ($p >$

0.05) amongst them. The presence of relatively higher proportion of gelatin against the drug in the formulation eventuated in supersaturation more quickly; wherefore, no further formulation was dissolved. The drug was enwrapped or encapsulated in the gelatin shell; thus, no further drug was dissolved too. For that reason, samples V, VI and VII containing relatively larger quantities of gelatin furnished ostensibly decreased solubility of piroxicam. The PLGN formulation IV exhibited apparently the highest solubility of piroxicam which was 600-times higher than that shown by PPDP (94.24 ± 25.65 µg/mL vs. 0.16 ± 0.21 µg/mL). Furthermore, solubility of piroxicam furnished by the corresponding physical mixture (PM) was 29.53 ± 24.34 µg/mL which was less than that shown by the optimized formulation. The PM was prepared by simply triturating piroxicam and gelatin in 1/8 (w/w) ratio using a pestle and mortar.

The piroxicam content in the optimized PLGN formulation was 93.41± 0.56 %. The high drug content was owing to the solvent-evaporation method. In this approach, all the constituents of a formulation are first entirely dissolved in a solvent system to get a transparent homogeneous mixture and then subjected to drying; thus, drug/excipient ratio is not affected even if some of the solution is spilt accidentally. This method also ensures high content uniformity as molecular level mixing is achieved when a clear solution is formed. Moreover, drug loading in the optimized formulation was 10.45 ± 0.06%.

The encapsulation efficiency calculated was 66.74 ± 6.87%. The optimized PLGN formulation was rinsed thrice with absolute ethanol because the free drug is soluble in absolute ethanol while gelatin is insoluble in absolute ethanol. Thus, free drug was percolated with filtrate and encapsulated drug remained preserved inside gelatin shell. Furthermore, 40% hydroalcoholic solution at 50°C was used for dissolving the residue and leaching of encapsulated drug because gelatin is soluble in 40% aqueous ethanol at 35-40°C [12].

The release rate of piroxicam from the PLGNs is shown in Fig. 2. All the formulations showed higher dissolution of the payload as compared to PPDP. The improved release of the drug from the formulations might be credited to enhanced wetting and conversion of piroxicam to the amorphous counterpart by gelatin. Gelatin hinders recrystallization of the drug from solution. The amorphous drug bears more surface area exposed to surrounding medium; accordingly, fostering the release consequently. Amongst all the trialed formulations in this study, PLGN formulation IV consisting of drug/gelatin (1/8, w/w) exhibited the most expedited release rate. In particular, the release rate was about 7-fold higher than that of PPDP (85.12 ± 10.96% vs. 11.81 ± 5.34%) and 3-fold as compared to PM (85.12 ± 10.96% vs. 29.56 ± 6.52%) in 10 minutes. The release profile of piroxicam pertaining to PM was erratic. The release rate of the drug was gradually decreased with increasing drug/gelatin ratio from 1/10 (w/w) to 1/20 (w/w) in formulations V to VII, respectively. This might be ascribed to the existence of relatively thicker gelatin shell around the core which a little bit delayed the drug release. Based on the highest apparent solubility and release rate, PLGN sample IV was opted for further characterization.

The degree of crystallinity was scrutinized using PXRD system and further confirmed by DSC. The PXRD pattern of PPDP represented its particular crystalline nature (Fig. 3A). The distinguishing spikes of piroxicam appeared at 8.58°, 11.73°, 14.54°, 16.69°, 17.62°, 18.78°, 21.76°, 22.48° and 27.40°. The absence of any sharp-pointed projection in the pattern of gelatin confirmed its amorphous nature (Fig. 3B). The distinctive barbs of piroxicam were also noticed in the pattern of PM (Fig. 3C) which suggested that the drug retained its crystalline disposition in PM. On the other hand, the spikes corresponding to piroxicam vanished in the pattern of PLGN formulation IV (Fig. 3D). This suggested that piroxicam lost its inherent crystalline property during the preparation of PLGNs and embraced amorphous one after loading. The conclusions

obtained by DSC analyses were in accord with those given by PXRD analyses. A sharp endotherm emulating the melting point of piroxicam was seen at 200°C in the thermogram of PPDP (Fig. 4A) which confirmed its particular crystalline disposition. Thermogram of gelatin was devoid of any sharp endotherm (Fig. 4B), yet, a wide curve steeping downward was seen in the range of 30-180°C which was owing to consumption of heat for vaporizing moisture present in the sample. A sharp endotherm emulating the melting point of the drug at 200°C and the broad curve steeping downward in the range of 30-180°C were seen in the thermogram of PM (Fig. 4C). This confirmed that piroxicam did not transmute its crystalline disposition in PM. On the other hand, both of these endothermic behaviours were not present in the thermogram of PLGN sample IV (Fig. 4D). This suggested that the encapsulated drug was in the amorphous state and the formulation prepared was completely dry. The conversion to the amorphous state might be ascribed to the influence of gelatin in the formulation and the preparation method.[24]

TGA thermograms recorded in the range of 50-550°C are revealed in Fig. 5. There is only 10% loss in weight up to 230°C due to escape of physically attached moisture. Approximately, 3% degradation of PPDP occurred in the range of 50-250°C (Fig. 5A). Then, 71% further sudden degradation of PPDP was observed in the range of 250-300°C. Overall, 88% PPDP was degraded in the range of 50-550°C. In the case of gelatin, 7% weight loss was observed in the range of 50-200°C (Fig. 5B). About 44% further degradation of gelatin was seen in the range of 200-300°C. In total, 67% degradation of gelatin took place in the range of 50-550°C. The PM showed 6% weight loss in the range of 50-180°C followed by 56% further weight loss in the range of 180-350°C, and total weight loss was about 69% in the range of 50-550°C (Fig. 5C). In the case of PLGN, only 1% weight loss occurred in the range of 50-180°C, next 40% weight loss took place in the range of 180-350°C, and 48% total weight loss was noticed in the range of 50-550°C (Fig.

5D). The comparison of TGA thermogram of PM with that of PLGN suggested that the encapsulated drug was thermally more stable. Gelatin (wall material) performs as a protective shield for the encapsulated products (core content) and thereby prolongs the shelf-life of the products.[13]

Scanning electron micrographs of PPDP (Fig. 6A) and gelatin (Fig. 6B) showed particles with irregular contours and surface features. Each round-shaped particle of PLGN formulation IV was bearing a dimple on one side and exhibited an unwrinkled surface (Fig. 6C). A pharmaceutical nanocapsule is a nano-sized (≤1000 nm) drug-loaded spherical particle having a core-shell morphology. From Fig. 6C, it can be observed that the particle-size of all the shown particles is less than 1000 nm. Thus, particles of electrosprayed PLGN formulation IV were nanocontainers. A smooth surface of the particles of PLGN and a core-shell morphology is shown in Fig. 6D.

FTIR spectra are shown in Fig. 7. The spectrum of PPDP revealed sharp distinctive spikes mainly in the fingerprint region at 452 cm^{-1}, 527 cm^{-1}, 560 cm^{-1}, 581 cm^{-1}, 611 cm^{-1}, 686 cm^{-1}, 733 cm^{-1}, 758 cm^{-1}, 825 cm^{-1}, 876 cm^{-1}, 936 cm^{-1}, 1145 cm^{-1}, 1180 cm^{-1}, 1345 cm^{-1}, 1435 cm^{-1} and 1529 cm^{-1}, and at 3338 cm^{-1}. Other peaks appeared at 1575 cm^{-1} and 1630 cm^{-1} (Fig. 7A). The absorption band appearing at 3338 cm^{-1} was owing to valence vibrations of hydroxyl groups (-OH) and secondary amide groups (-NH).[25,26] Absorption bands corresponding to the conjugating benzene and pyridine rings are situated in the range of 1650-1550 cm^{-1}.[27] The band appearing at 1630 cm^{-1} was due to valence vibrations of carbonyl group (C=O) of the secondary amides while a high intensity band at 1529 cm^{-1} was because of deformation vibrations of amide groups (-NH). The bands located at 1575 cm^{-1} and 1435 cm^{-1} are due to valence vibrations (C=C) of benzene and pyridine rings. The presence of sulphur dioxide (SO_2) group in piroxicam was confirmed by the bands located at 1345 cm^{-1} and 1180 cm^{-1} arising from the asymmetric and symmetric valence

vibrations, respectively. The spikes appearing at 876 cm^{-1}, 825 cm^{-1} and 758 cm^{-1} were characteristic peaks of deformation vibrations in the plane (C-H) of aromatic rings present in piroxicam. FTIR spectrum of gelatin is shown in Fig. 7B. All the distinctive spikes of piroxicam mentioned above were also witnessed at the same positions in both the spectra of PM (Fig. 7C) and PLGN formulation IV (Fig. 7D). Moreover, the spectrum of PM was identical to that of the formulation IV; therefore, this suggested non-existence of interactions between piroxicam and gelatin.

Figure 7 reveals the mean piroxicam concentration-time graphs constructed with the data regarding oral administration of PPDP (Fig. 8A) and PLGN formulation IV (Fig. 8B). The formulation resulted in higher mean plasma concentrations of piroxicam at all time intervals between 1-48 h (*t*-test, $p < 0.05$) than did PPDP. The pharmacokinetic attributes, such as AUC, C_{max} and T_{max}, are displayed in Table 2. The AUC and C_{max} with formulation IV were significantly ameliorated as compared to those obtained with PPDP (*t*-test, $p < 0.05$); nevertheless, T_{max} did not alter significantly (*t*-test, $p > 0.05$). As compared with PPDP, bioavailability was ~ 4-fold with PLGN formulation IV. The better bioavailability can be credited to augmented solubility and dissolution of piroxicam due to improved wetting by gelatin, nanosizing and transmutation of the inherent crystalline structure of the drug to the amorphous one. Thus, this formulation consisting of piroxicam/gelatin (1/8, w/w) might be an important bioavailability-enhancing system for delivery of piroxicam orally.

Conclusion

PLGN formulation IV, consisting of piroxicam and gelatin at the ratio of 1/8 (w/w), provided the most boosted apparent solubility (94.24 ± 25.65 µg/mL) and release rate (85.12 ± 10.96% in

10 min). The solubility was 600-times higher than that of PPDP and 3-fold as compared to PM. Moreover, release rate was 7-fold and 3-fold higher, respectively. The drug content, drug loading and encapsulation efficiency of this formulation were 93.41± 0.56%, 10.45± 0.06% and 66.74± 6.87%, respectively. The encapsulated amorphous piroxicam exhibited more stability, and had no covalent linkage with gelatin. The reinforcement in solubility and dissolution of piroxicam in water can be owing to improved wetting of piroxicam due to the presence of gelatin and conversion of piroxicam from its crystalline structure to the amorphous one in PLGN. The amelioration in dissolution rate and solubility of piroxicam in PLGN lead to greater bioavailability of piroxicam. As compared with PPDP, enhancement in bioavailability with PLGN formulation IV was 4-fold. Thus, this formulation is a prospective bioavailability enhancing drug delivery system for administeration of piroxicam via the oral routre.

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Table 1. Compositions of PLGNs.

Constituents (w/w)	I	II	III	IV	V	VI	VII
Piroxicam	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Gelatin	1.0	2.0	4.0	8.0	10.0	15.0	20.0

PLGNs = piroxicam-loaded gelatin-nanocontainers.

Table 2. Pharmacokinetic parameters obtained after oral administration.

Parameter	PLGN formulation IV	PPDP
$AUC_{(0-\infty)}$ (h·μg/ml)	971.13 ± 185.74*	251.42 ± 214.10
C_{max} (μg/ml)	61.04 ± 15.38*	15.13 ± 8.88
T_{max} (h)	5.00 ± 0.00	5.00 ± 1.00
$t_{1/2}$ (h)	10.40 ± 2.85	15.28 ± 3.00
K_{el} (h^{-1})	0.07 ± 0.01	0.05 ± 0.01
$MRT_{(0-\infty)}$ (h)	16.02 ± 1.42	17.73 ± 9.39

Each value represents the mean ± SD (n=6). *P<0.05 compared with PPDP.

PLGN = piroxicam-loaded gelatin-nanocontainer; PPDP = piroxicam plain drug powder.

Figure Legends

Fig. 1. Effect of composition on the aqueous solubility of piroxicam in piroxicam-loaded gelatin-nanocontainers (PLGNs). Solubility of piroxicam plain drug powder (PPDP) and physical mixture (PM) in water is also shown. Each value shows the mean ± SD (n=3).

Fig. 2. Effect of composition on release rate of piroxicam from piroxicam-loaded gelatin-nanocontainers (PLGNs). Dissolution with piroxicam plain drug powder (PPDP) and physical mixture (PM) is also shown. Each value shows the mean ± SD (n=6).

Fig. 3. PXRD patterns: (A) piroxicam plain drug powder (PPDP), (B) gelatin, (C) physical mixture (PM) and (D) piroxicam-loaded gelatin-nanocontainers (PLGNs).

Fig. 4. DSC thermograms: (A) piroxicam plain drug powder (PPDP), (B) gelatin, (C) physical mixture (PM) and (D) piroxicam-loaded gelatin-nanocontainers (PLGNs).

Fig. 5. TGA thermograms: (A) piroxicam plain drug powder (PPDP), (B) gelatin, (C) physical mixture (PM) and (D) piroxicam-loaded gelatin-nanocontainers (PLGNs).

Fig. 6. SEM images: (A) piroxicam plain drug powder (PPDP) (× 5000), (B) gelatin (× 300), (C) piroxicam-loaded gelatin-nanocontainers (PLGNs) (× 15,000) and (D) core-shell morphology of PLGNs.

Fig. 7. FTIR spectra: (A) piroxicam plain drug powder (PPDP), (B) gelatin, (C) physical mixture (PM) and (D) piroxicam-loaded gelatin-nanocontainers (PLGNs).

Fig. 8. Piroxicam mean titer in plasma versus time graphs: (A) piroxicam plain drug powder (PPDP) and (B) piroxicam-loaded gelatin-nanocontainers (PLGNs). *$P<0.05$ as compared to PPDP.

Figure 1

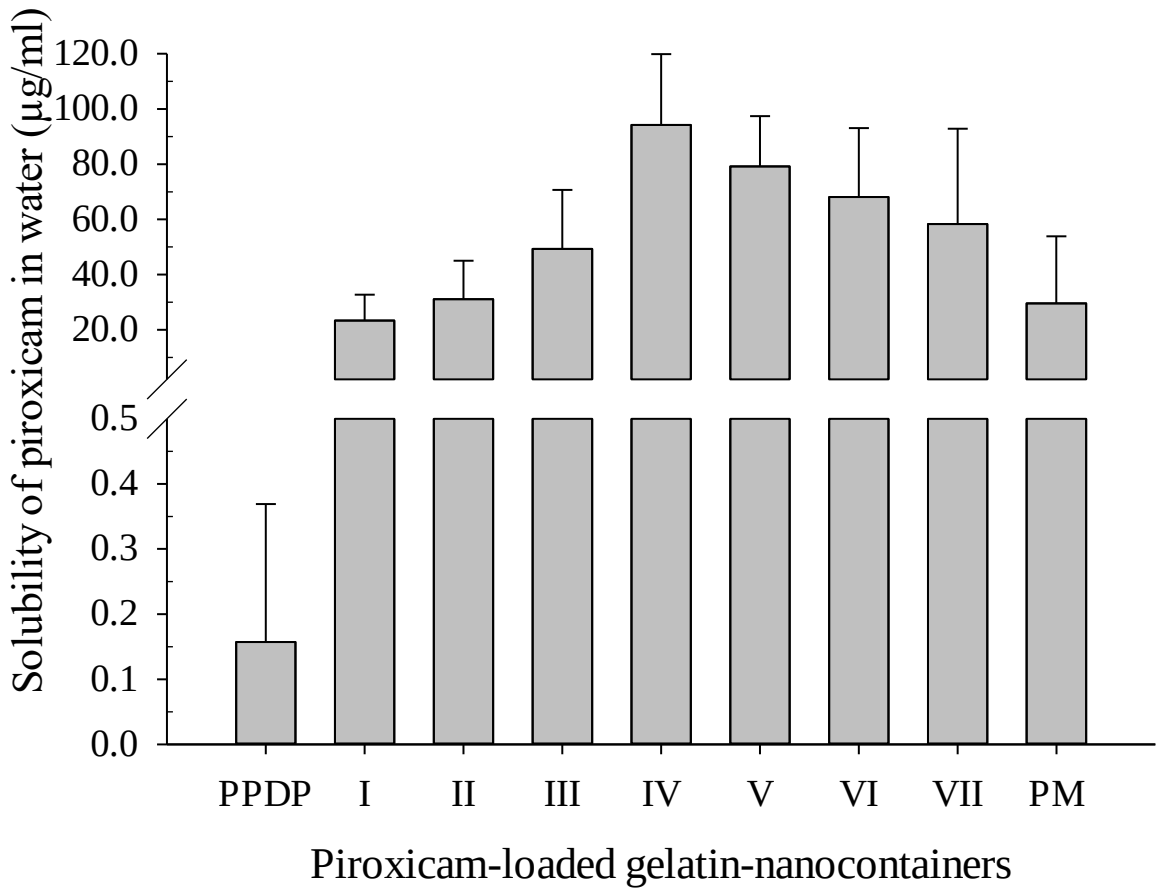

Solubility of piroxicam in water (µg/ml)
120.0
100.0
80.0
60.0
40.0
20.0
0.5
0.4
0.3
0.2
0.1
0.0
PPDP
I
II
III
IV
V
VI
VII
PM
Piroxicam-loaded gelatin-nanocontainers

Figure 2

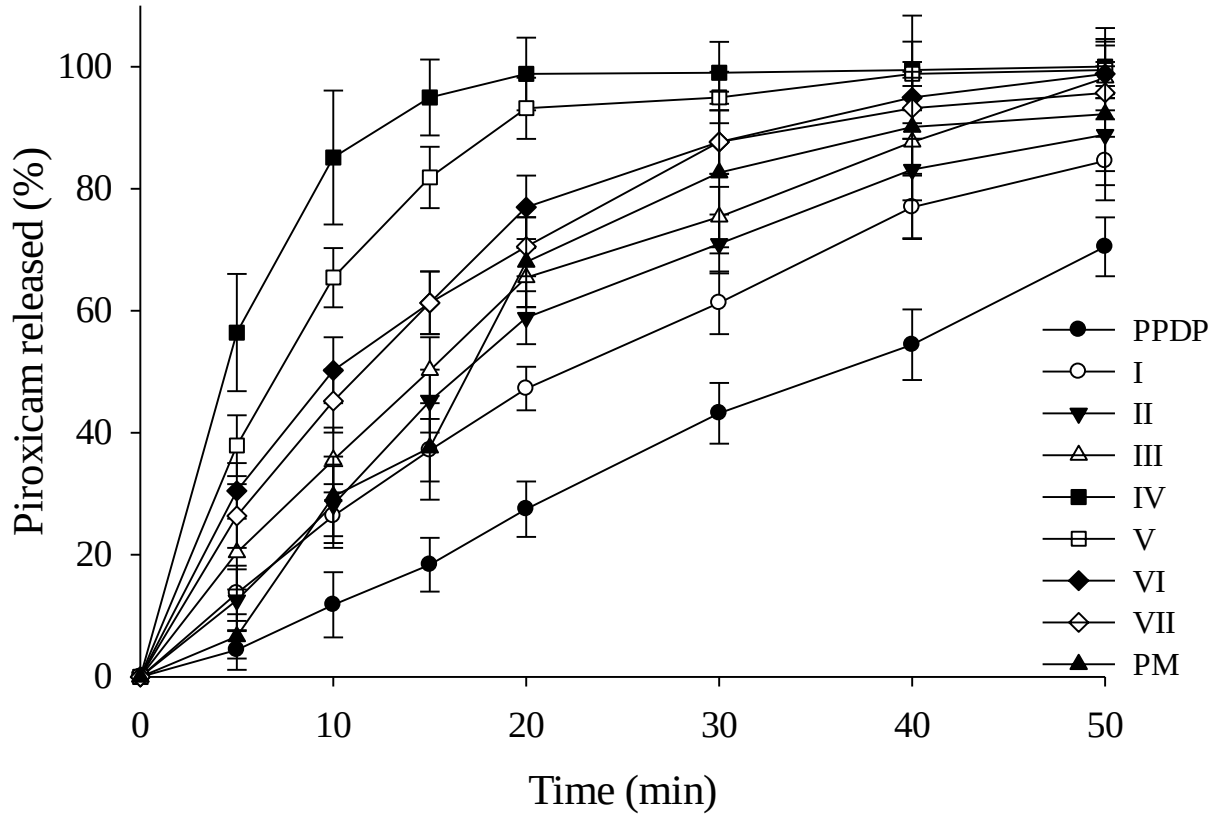

Piroxicam released (%)
100
80
60
40
20
0
0
10
20
30
40
50
Time (min)
PPDP
I
II
III
IV
V
VI
VII
PM

Figure 3

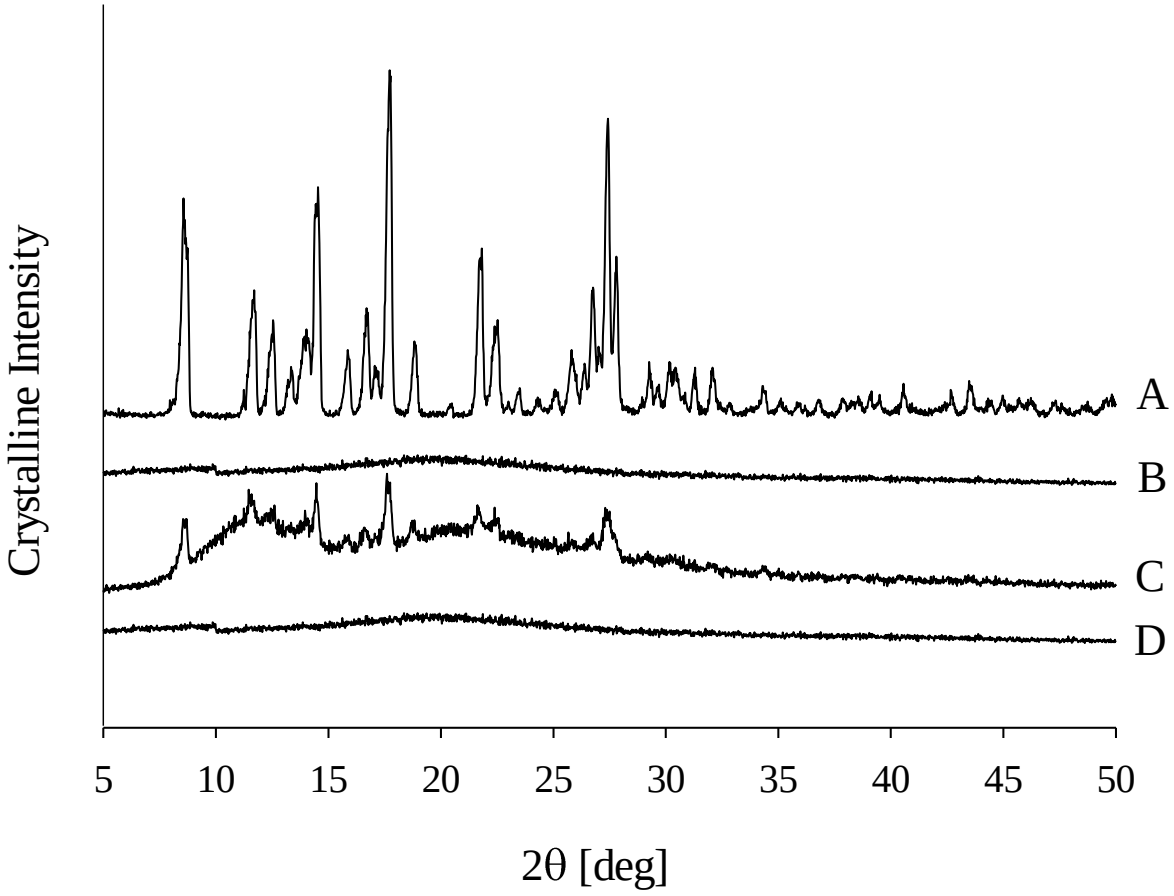


Figure 4

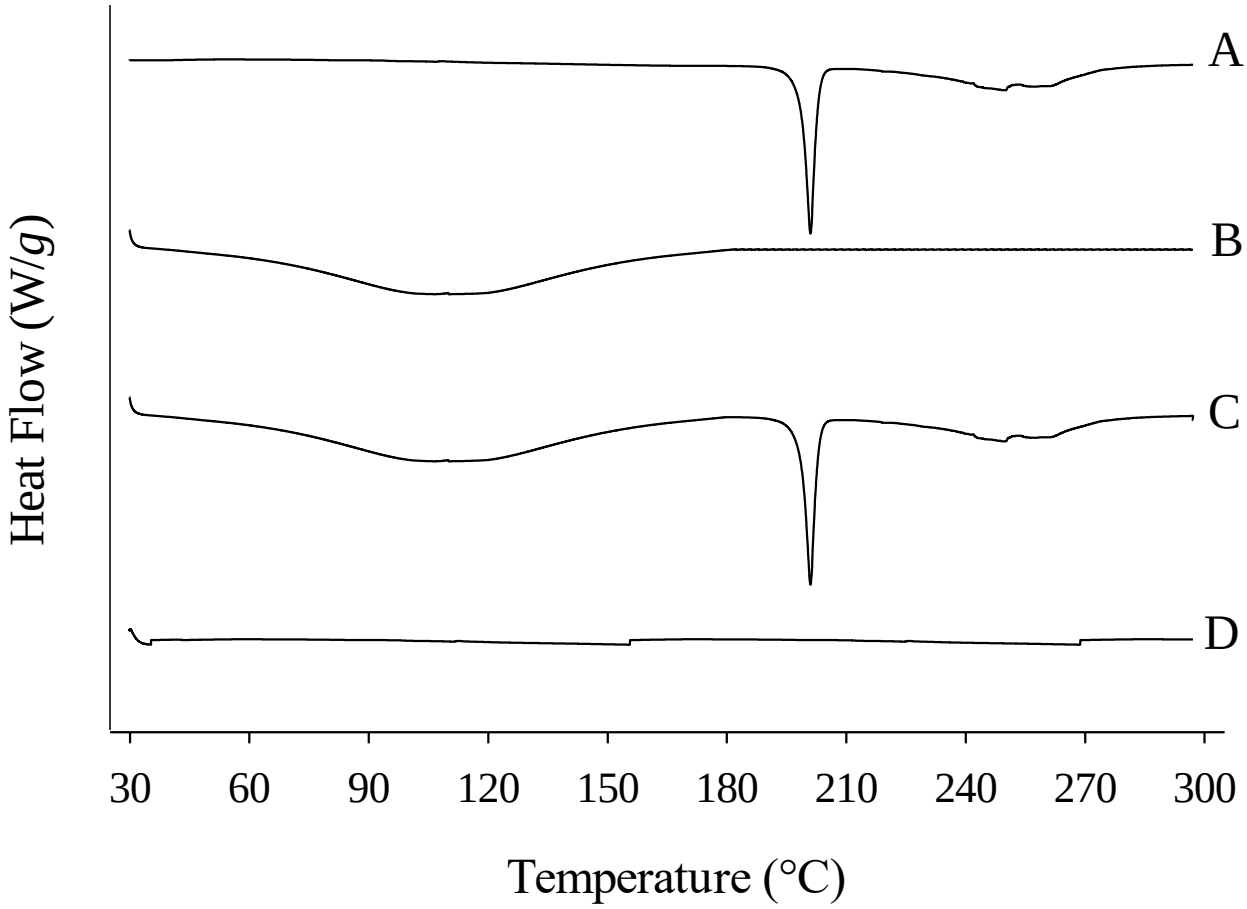

A
B
C
D
Heat Flow (W/g)
30
60
90
120
150
180
210
240
270
300
Temperature (°C)

Figure 5

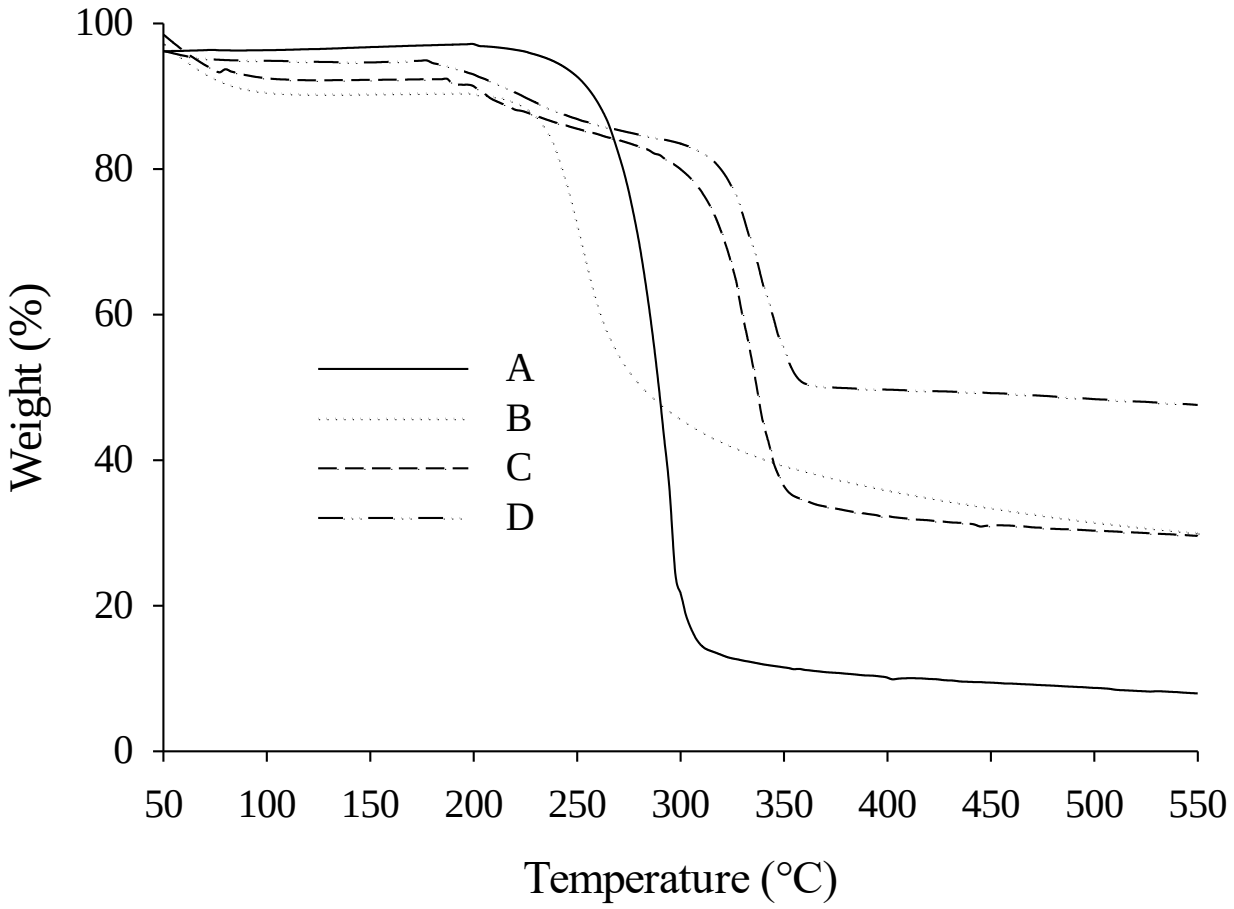

100
80
60
40
20
0
Weight (%)
A
B
C
D
50
100
150
200
250
300
350
400
450
500
550
Temperature (°C)

Figure 6A

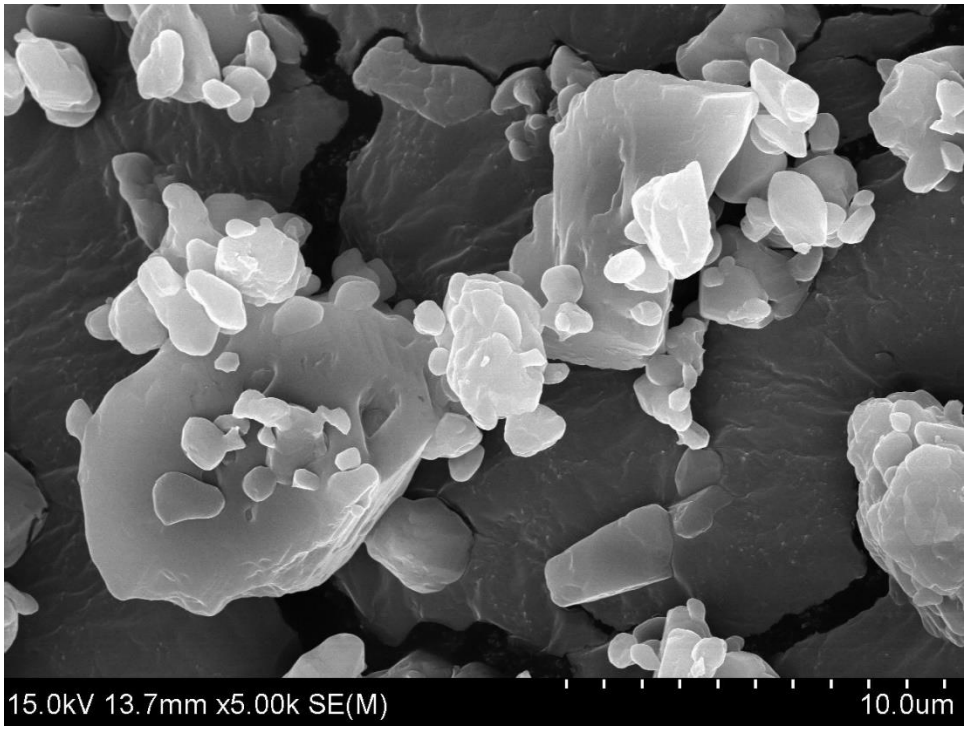


Figure 6B

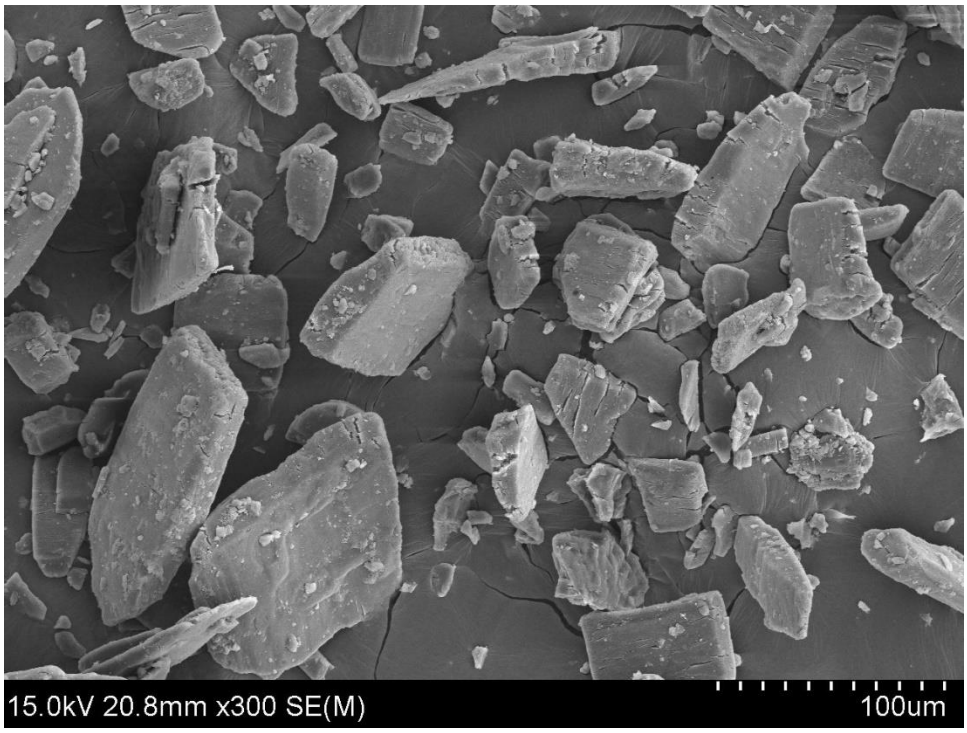


Figure 6C

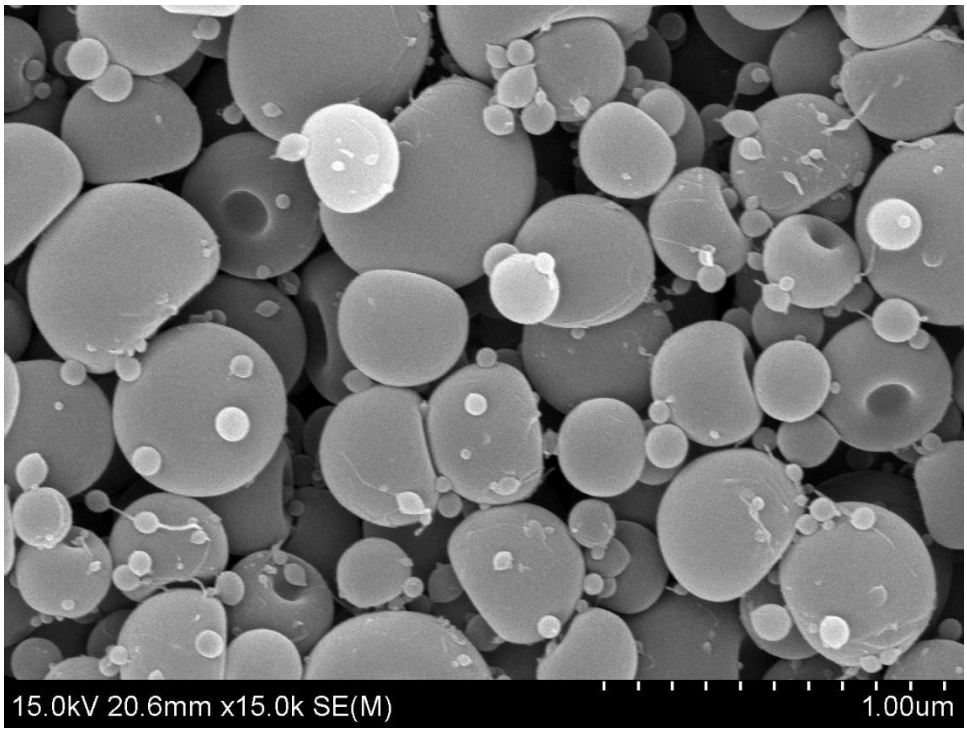


Figure 6D

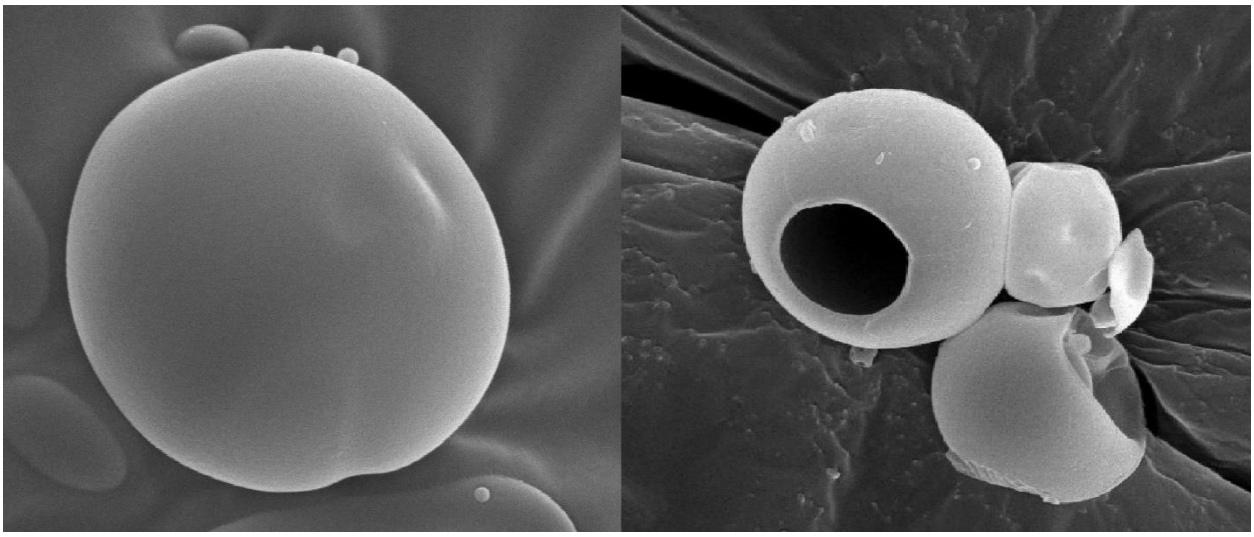


Figure 7

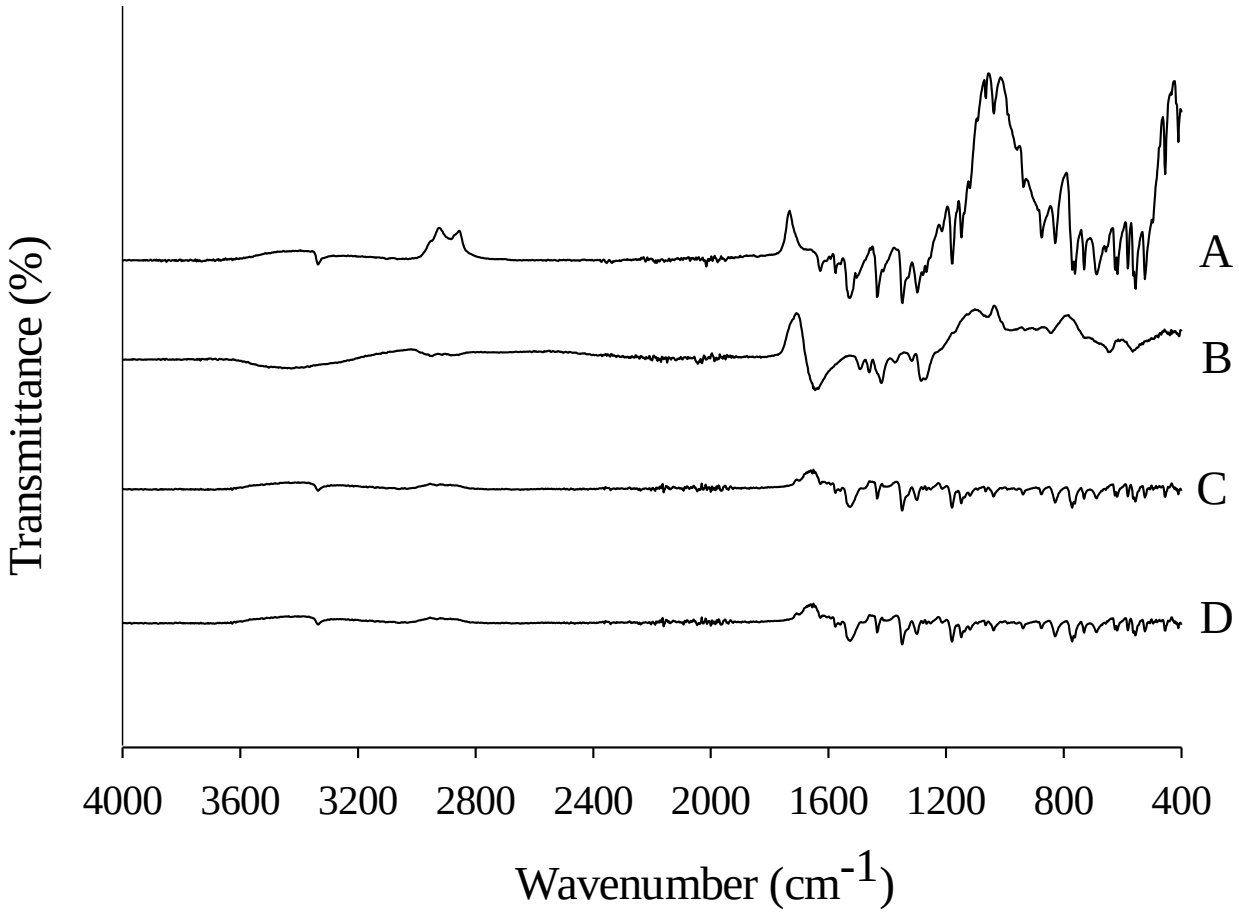
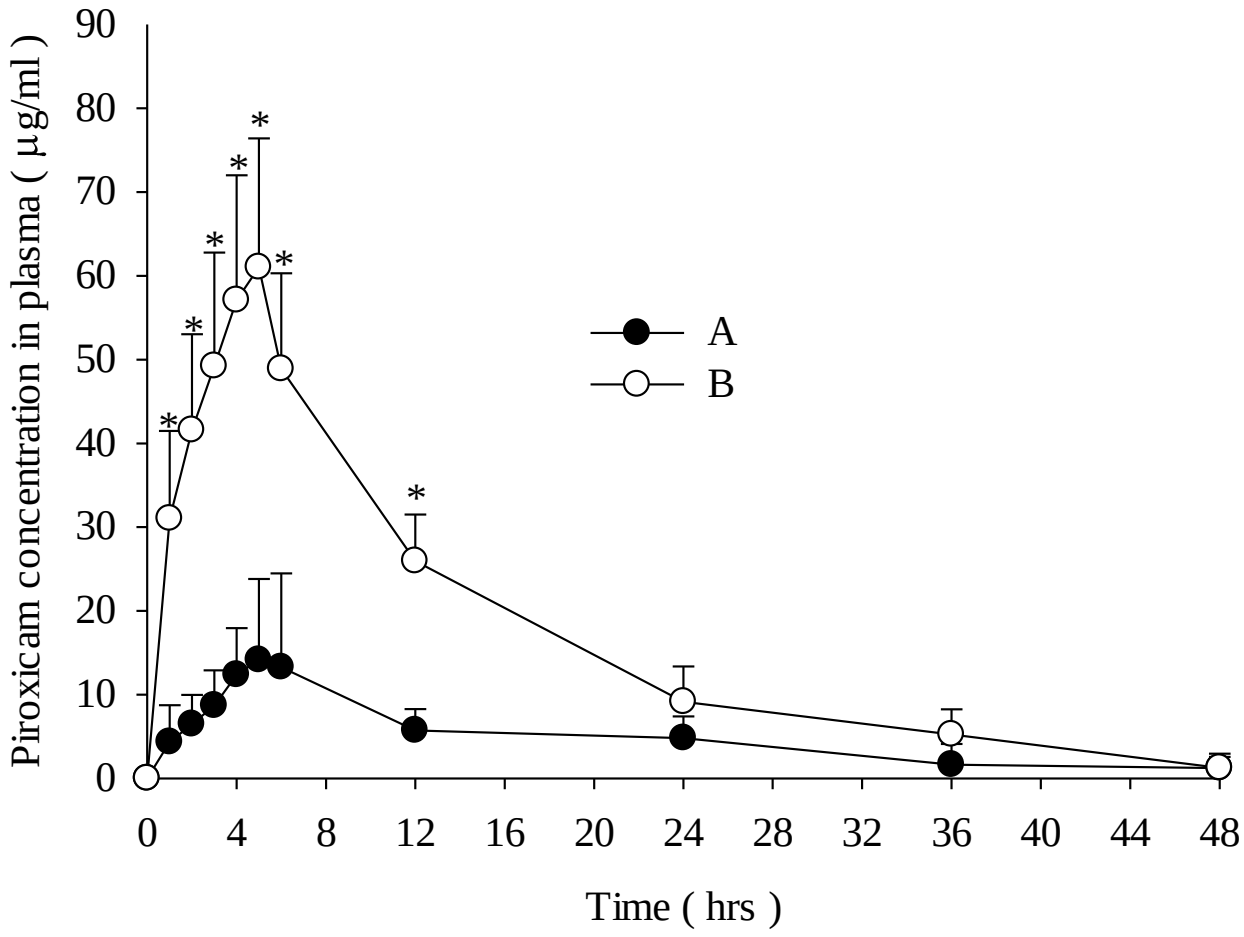

Transmittance (%)
A
B
C
D
4000
3600
3200
2800
2400
2000
1600
1200
800
400
Wavenumber (cm-1)

Figure 8


Piroxicam concentration in plasma (μg/ml)
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
32
36
40
44
48
Time (hrs)
A
B
*